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SHORT COMMUNICATION

Minimal variation of the plasma lipidome after delayed processing of neonatal cord blood

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Abstract

Background Cord blood lipids are potential disease biomarkers. We aimed to determine if their concentrations were affected by delayed blood processing.

Method Refrigerated cord blood from six healthy newborns was centrifuged every 12 h for 4 days. Plasma lipids were analysed by liquid chromatography/mass spectroscopy.

Results Of 262 lipids identified, only eight varied significantly over time. These comprised three dihexosylceramides, two phosphatidylserines and two phosphatidylethanolamines whose relative concentrations increased and one sphingomyelin that decreased.

Conclusion Delay in separation of plasma from refrigerated cord blood has minimal effect overall on the plasma lipidome.

Keywords Plasma lipidomics · Cord blood · Delayed processing · Reproducibility · Day-to-day variability

Birth cohort studies have incorporated cord blood collection and storage for disease biomarker discovery (Janssen et al. 2017; Penno et al. 2013; Vuillermin et al. 2015; Lee et al. 2014). Targeted lipidomics is a promising tool for biomarker discovery (Meikle et al. 2014) but whether delay in blood processing, which is common when samples are shipped to a central laboratory (Penno et al. 2016), is a source of variation is not well documented. We aimed therefore to determine whether delays in cord blood processing affected the observed concentrations of plasma lipids.

With ethical approval from Walter and Eliza Hall Institute Human Research Ethics Committee and written informed consent from mothers of newborns, cord blood samples of 55–120 mL in 21 mL of citrate anticoagulant were obtained from six healthy infants (4M, 2F) born at between 35 and 41 weeks' gestation. Samples were transferred to the laboratory within 4 h of collection, aliquoted into 1.5 mL Eppendorf tubes and placed at 4 °C. Approximately every 12 h for 4 days the tubes were inverted 5 times, centrifuged and the plasma stored at − 80 °C.

Lipid was extracted by adding 100 µL 1:1 butanol:methanol spiked with internal lipid standards (PC19:0/19:0, PE-d31, PG17:0/17:0, DG-d5 and TG-d5) to 10µL plasma, mixing for 1 h at room temperature then sonicating and centrifuging at 17000×*g* for 10 min (Alshehry et al. 2015). A small amount of each sample supernatant was pooled to create 16 internal quality controls for loading at regular intervals during the run. Each supernatant was injected into an Agilent (Santa Clara, CA) 2.7µ m Poroshell column with isopropanol:acetonitrile:water solvent and analysed by LC ESI-MS/MS using an Agilent 1290 liquid chromatography system and 6490 triple quadrupole mass spectrometer as previously described (Huynh et al. 2018). Data processing using Agilent Mass Hunter software identified 265 lipids. Raw data for each lipid are provided as supplemental data. The coefficient of variation for each of the five internal standards within five external adult plasma quality control samples was less than 8%.

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Three lipids whose CVs exceeded 20% in the internal quality control samples were excluded. Peak areas for the remaining 262 lipids were then normalised to internal standards (lipid-standard pairs provided as supplemental information) and $\log_2$-transformed and scaled to mean and standard deviation. Principal component analysis (PCA) to determine variation due to individual and processing delay was performed using the Phyloseq package within R software (v1.18.0; http://www.r-project.org). The paired LIMMA test with 5% false discovery rate (Gentleman et al. 2004) was used to identify significant differences in lipid abundance relative to the baseline sample, defined as an adjusted p value < 0.05.

PCA demonstrated that refrigerated storage for more than 90 h had minimal impact on intra-individual lipidomic reproducibility (Fig. 1a). Individual differences between the six newborns accounted for 97.6% of the variability whereas delay in processing accounted for just 0.6%. Eight of the 262 lipids changed significantly from baseline at one or more of the 8 subsequent time points. All three dihexosylceramide (DHC) and both phosphatidylserine (PS) lipids progressively increased by at least twofold over baseline after 90 h. Smaller changes were observed for two of the five plasmenylethanolamine (PE (P)) lipids and one of the 29 sphingomyelin (SM) lipids (Fig. 1b).

Thus, only a minority of the 262 cord plasma lipids identified by targeted lipidomic profiling showed significant variation after delayed processing and storage at 4 °C. This is consistent with a prior study that described delayed processing of EDTA blood stored on ice for 6 h did not alter concentrations of 15 lipids that were also identified using our method (Kamlage et al. 2014). Nonetheless, storage of blood at room temperature for more than 2 h increases lysophospholipid concentrations and, to a much greater extent, changes the concentrations of amino acids, carbohydrates and signaling molecules (Kamlage et al. 2014, 2018).

The progressive changes in the concentrations of all measured PS and DHC lipids are likely to reflect specific biological processes. For example, PS is a marker of platelet activation (Lentz 2003) and apoptotic cell death (Schutters and Reutelingsperger 2010), which are expected to increase over storage time. DHC, existing mostly as lactosylceramide in humans, can be produced by the action of lactose synthase in blood (Yamato and Yoshida 1982), potentially explaining the increase in DHC over time.

In summary, delayed processing of refrigerated cord blood has minimal impact overall on its plasma lipidomic profile. Nevertheless, time-dependent changes in the 8 variable lipids may have implications for biomarker discovery. The increases in PS and DHC are also potentially relevant for cord blood-based immune studies because PS is a broad-spectrum immunosuppressant (Birge et al. 2016) and lactosylceramide (DHC) promotes cell proliferation, migration and adhesion (Chatterjee and Pandey 2008).

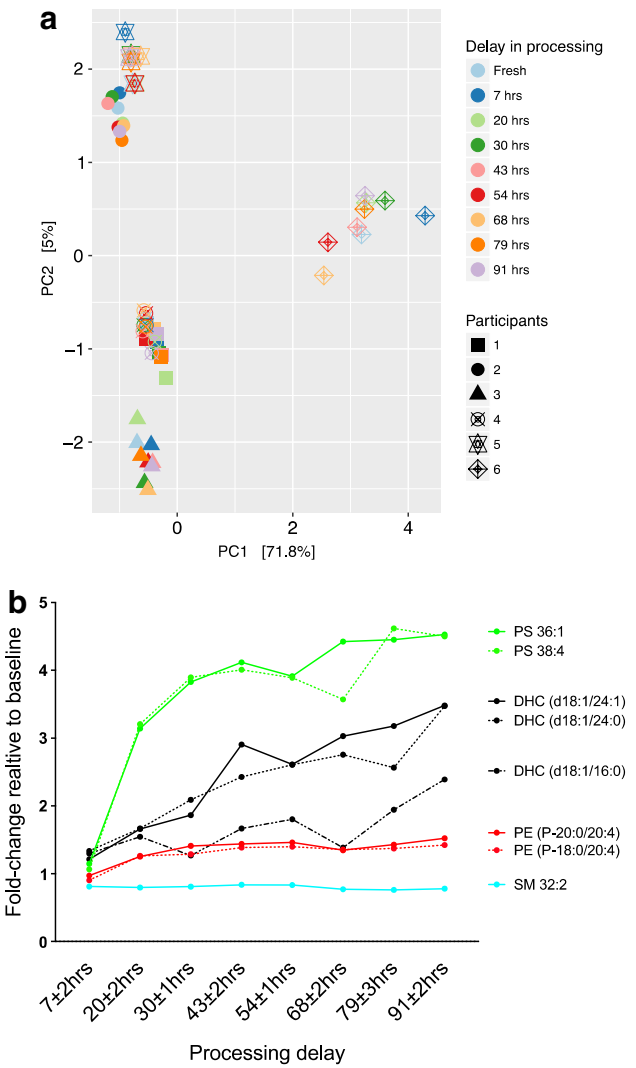

Fig. 1 Prolonged storage of cord blood at 4 °C affects a minority of plasma lipids identified by targeted lipidomics. **a** Principal components analysis of lipidomes of cord plasma collected from six healthy newborns and stored for up to 96 h at 4 °C before processing and storage. **b** Changes over time in the relative abundance of lipids that varied significantly at one or more time point compared to baseline. The mean ± SD processing delay for the six samples is indicated. *PS* phosphatidylserine; *PE (P)* plasmenylethanolamine; *DHC* dihexosylceramide; *SM* sphingomyelin

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Author contributions The study was devised by JMW, MASP and LCH, who with EB-S arranged sample collection and storage. Lipid data were generated by KK and analysed by KK and NGB. JMW and LCH drafted the manuscript and all authors contributed to the final version.

Compliance with ethical standards

Conflict of interest No author has a conflict of interest to declare.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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